there is added with stirring 1 n mole of sodium phthalate. After a 5 minute incubation at a temperature of 37° C. freshly prepared guinea pig complement is added and the reaction is allowed to proceed for 30 minutes at a temperature of 37° C. At the end of this period an equal volume of 100 mM tris/HCL buffer (pH 8.0) containing 1% Triton X-100, supra., is added and the absorbance is immediately read at 405 nm in a spectrophotometer against a blank treated in the same way as the test and consisting of all the reagents except the sodium phthalate. The difference between the blank and the test is a measure of the amount of analyte present in the unknown and can be quantitated by reference to a standard curve. The absorbance increases in a dose dependent fashion with increasing amounts of phthalate. A wide variety of enzymes can be encapsulated in the liposome to serve as the amplification system. The enzymes are used with the appropriate substrates as is readily apparent to those skilled in the art (see for example U.S. Pat. No. 4,193,983).

EXAMPLE 7

Freshly separated lipid vesicles from Preparation 5, supra., was incubated with antiphthalate monoclonal antibody (1 mg/ml) that previously had between 2–4 reactive sulfhydryl groups placed on them by the method of Carlsson et al, Biochem. J., 173, 723–737 (1978). The incubation was done in a total volume of 21 ml in the phosphate buffer pH 6.0 for 12 hours at room temperature under a blanket of nitrogen gas. Lipid vesicles containing the antiphthalate antibody were separated from the unattached antibody by column chromatography on agarose gels in 100 mM sodium chloride—10 mM sodium phosphate (pH 7.4). Under these conditions approximately 175 μg protein are coupled to a 1 μmole of lipid.

Incubation of 5 nmoles of lipid vesicles containing the antibody in 0.2 ml of 100 mM sodium chloride-2 mM histidine-1,5 mM calcium chloride at 37° C. in the presence of phthalate and fresh complement for 15 minutes results in the lysis of the vesicle and the release of calcein. The extent of lysis increases with increasing concentrations of phthalate. Incubation of the vesicles in the absence of either phthalate or complement results in no lysis of the vesicles and no increase of the signal from calcein.

EXAMPLE 8

ATTACHMENT OF ANTIBODY FOLLOWING GENERATION OF ALDEHYDE ON THE VESICLE BY TREATMENT WITH GALACTOSE OXIDASE

A portion of the lipid vesicles prepared according to the procedure of Preparation 1, supra., encapsulating carboxyfluorescein was provided.

To attach the antibody to the vesicle surface the C-6 hydroxyl group on the galactose residue of the lactosylcerebroside was oxidized to an aldehyde by the enzyme galactose oxidase as detailed by Zile et al, J. Biochem., 254,3 547-3553 (1979). 5 μmoles of the vesicle lipid was incubated with 25 units of galactose oxidase (Sigma Corp.) in saline for 4 hours at a temperature of 37° C. Lipid vesicles containing an aldehyde group on their surface are separated from the enzyme by column chromatography. The fraction containing the vesicles was mixed with 2 mg of antiphthalate antibody and 0.2 ml of a freshly prepared solution of sodium cyanoborohydride and allowed to react at room temperature overnight. The vesicles containing the antibody on their surface are separated from non-attached antibody. The resulting vesicles are suitable for use in the immunoassay described in Example 2.

EXAMPLE 9

PREPARATION OF A UNIVERSAL IMMUNOASSAY REAGENT

A rabbit antimouse antibody was treated with pepsin to form an $F(ab')_2$ fragment. This fragment retains the two antimouse antibody binding sites but does not have the region that is recognized by complement. The fragment was incubated for 90 min. with 20 mM dithiothreotol in 50 mM sodium acetate pH 5.5 under nitrogen gas at room temperature. The resulting Fab' fragments were separated from the dithiothreitol on a Sephadex G-25 column and immediately mixed with lipid vesicles composed of MBPE/phosphatidylcholine/cholesterol: 1/9/8 as described in Preparation 5, supra., and incubated for 12 hours at room temperature and under a blanket of nitrogen gas. The vesicles with rabbit antimouse Fab' fragments attached can now be mixed with any mouse antibody to form an immunoassay reagent. 50 nmoles of lipid vesicle containing 4 μg of rabbit antimouse Fab' fragments were incubated for 30 minutes with 50 μg of mouse antiphthalate monoclonal antibody in 1 ml of saline. Mouse antibody that did not become attached to the vesicle was separated from the vesicle antibody complex by centrifugation at 12,000×G for 15 minutes. The vesicle antibody complex is resuspended in 1 ml of 0.1 M sodium chloride, 2 mM histidine 1 mM calcium chloride and 100 μl of the vesicles is mixed with 10 μl of the phthalate solution and 15 μl of complement for 15 minutes. At the end of this period lysis is quantitated by the release of the calcein and is proportional to the amount of phthalate added (from 0.1 to 2 nmoles). In the absence of complement no increase in lysis will be observed.

In a similar fashion assays for other organic ligand substances can be prepared by adding mouse antibodies with binding sites for other antigens. A wide range of anti-immunoglobulin Fab' fragments such as the F(ab') and $F(ab')_2$ fragments can be prepared and used to form a flexible immunoassay procedure.

EXAMPLE 10

ATTACHMENT OF ANTIBODY TO THE LIPID VESICLES THROUGH A TETRADECYLMELIBIONAMIDE ACTIVATED BY 1,1'-CARBONYDIIMIDAZOLE TO FORM AN IMMUNOASSAY REAGENT

Tetradecylmelibionamide (1 mmole) synthesized as described by Williams et al., Arch. Biochem. Biophys., 195, 145–151 (1979) was placed in acetone and activated by adding 1 m mole of 1,1-carbonyldiimidazole as described in Bethell et al., J. Biol. Chem., 254, 2572–2574 (1979). Then 10 nmoles of the activated reagent was mixed with 1 mg of mouse antiphthalate antibody in 1 ml of 50 mM sodium phosphate-50 mM sodium chloride and allowed to incubate overnight at room temperature. The reaction mixture was then incubated with 5 μmoles of lipid vesicles composed of phosphatidylcholine/-phosphatidylethanolamine/cholesterol (9/1/5) containing 100 mM of calcein prepared as described in U.S. Pat. No. 4,235,871.